DEVELOPMENT OF A WHOLE MITOCHONDRIAL GENOME SEQUENCING STRATEGY TO MAXIMISE DISCRIMINATION POWER FOR INDIVIDUALS FROM LOW GENETIC DIVERSITY SUBPOPULATIONS.

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New Zealand (NZ) is situated in the South Pacific. The main subpopulations are Caucasians (mostly European or Middle Eastern), Eastern Polynesians (including NZ Maori, Cook Islanders, Tokelauans, Hawaiians or Tahitians), Western Polynesians (Samoans, Tongans or Niueans) or Asians (mostly Chinese, Korean, Japanese, or Filipino). Genetic diversity within individual Pacific Island populations is low due to migratory founder effects. Polynesian mitochondrial (mt) haplotypes include a 9bp deletion plus three temporally embedded substitutions. This Polynesian motif is now almost fixed in the peoples of Polynesia. Traditional forensic mtDNA analysis involving Sanger sequencing of the control region has not been implemented previously at ESR due to costs associated with maintaining a purpose-built facility and a labour intensive workflow, together with poor discrimination power for Pacific Island & Maori subpopulations. With the development of a sequencing facility & the availability of third party analysis software for forensic applications, we are re-evaluating the costs & discrimination potential of whole mtGenome testing using massively parallel sequencing (MPS).

An evaluation was undertaken using a strategy designed to minimise errors from PCR and library preparation chemistries to enable reliable detection of low-level heteroplasmy for reference-type samples, including buccal samples on both FTA card & swabs, & NIST SRM 2392 for which verified sequence data is available. Briefly, samples were either extracted using DNA IQ™ (Promega) followed by quantification of mtDNA, or amplified directly. Singleplex amplification of two overlapping amplicons was undertaken using PrimeSTAR® GXL DNA polymerase (TaKaRa Clontech), a hot start, long PCR polymerase recommended for high fidelity & mtDNA. Amplicons were prepared for sequencing using the PCR-free HyperPlus library preparation kit (Kapa) to reduce amplification bias & coverage dips. Libraries were sequenced on a MiSeq FGx™ (Illumina) & analysis was undertaken using GeneMarker®HTS software (SoftGenetics), blocking potential health information sequences. Direct long-range PCR of FTA card samples was successful. Analysis reports provide mitotype data and haplotypes suitable for subsequent phylogenetic verification in EMPOP. We conclude that this strategy is a useful option for any jurisdiction with low-diversity subpopulations. A population study will be progressed to characterise the discrimination potential for NZ subpopulations.